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First Record of *Aridelus rufotestaceus* Tobias (Hymenoptera: Braconidae, Euphorinae) Parasitizing *Nezara viridula* Nymphs (Heteroptera: Pentatomidae) with Observations on its Immature Stages and Development

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Abstract.—*Aridelus rufotestaceus* Tobias is recorded for the first time from Italy as parasitizing the pentatomid bug *Nezara viridula* L. This is the first record of the species in southern Europe and the first host data. The species is re-described and illustrated. New information is provided on its immature stages, development, and biological control potential.

Species of the euphorine braconid genus *Aridelus* Marshall are cosmopolitan in distribution, but most diversified in tropical areas (Shaw 1985). *Aridelus* species are quite distinctive in appearance, and can be easily distinguished from other braconids by their coarse honey-combed areolate mesosomal sculpture in combination with the long, tubular first metasomal segment and fore wing with a closed second submarginal cell (Shaw 1997). Their biology is not well known but the available records indicate that they are solitary koinobiont endoparasitoids of heteropteran bugs in the families Pentatomidae, Plataspidae, Scutelleridae, and Acanthosomatidae (Kirkpatrick 1937; Shenefelt 1969; Papp 1974; Čapek and Davidová-Vilimová 1978; Tobias 1986; Shaw 1988; Maetô and Kudô 1992).

Papp (1965) provided a taxonomic monograph of the world species of *Aridelus*, however, six Afrotropical species described by De Saeger (1946) were not included in Papp's monograph. Later, Papp (1974) erected the genus *Arideloides* for a species from New Guinea, but Shaw (1985) transferred the species to *Aridelus*. He (1980) described a new species from China, Chou (1987) revised the species of Taiwan, and, most recently, Chen and van Achterberg (1997) revised the *Aridelus* species of China. They indicated that about 40 *Aridelus* species are now known, of which 20 are recorded from China. Shaw (1985) estimated that there are at least ten undescribed *Aridelus* species in the Neotropical region. Despite recent taxonomic work, until now only one species of *Aridelus* has been recorded from Europe (Shenefelt 1969; Papp 1974; Čapek and Davidová-Vilimová 1978).

The purpose of this paper is to provide new host and distribution records for *Aridelus rufotestaceus* Tobias recently discovered in Italy parasitizing the pentatomid bug *Nezara viridula* (L.). This is the first record of the species in southern Europe and the first host data. The host, *Nezara viridula*, is one of the most serious agricultural insect pests worldwide, damaging a wide variety of fruit, nut, grain, and vegetable crops. It is the primary pest of soybean in many parts of the world (Todd 1989) and it also attacks many wild hosts that serve as reservoirs until agricultural crops are

available (Jones and Sullivan 1988). In Italy, annual losses due to this pest fluctuate in relation to changes in population abundance of *N. viridula* (Zandigiacomo 1990; Colazza and Bin 1990, 1995). Although this pest has been the focus of numerous biological control programs, most of the recorded biological control agents attack the egg stage. The only other parasitoids known to attack the nymphal and adult stages of *N. viridula* are Tachinidae and Encyrtidae. Until now no species of Braconidae has been discovered attacking this host (Jones 1988).

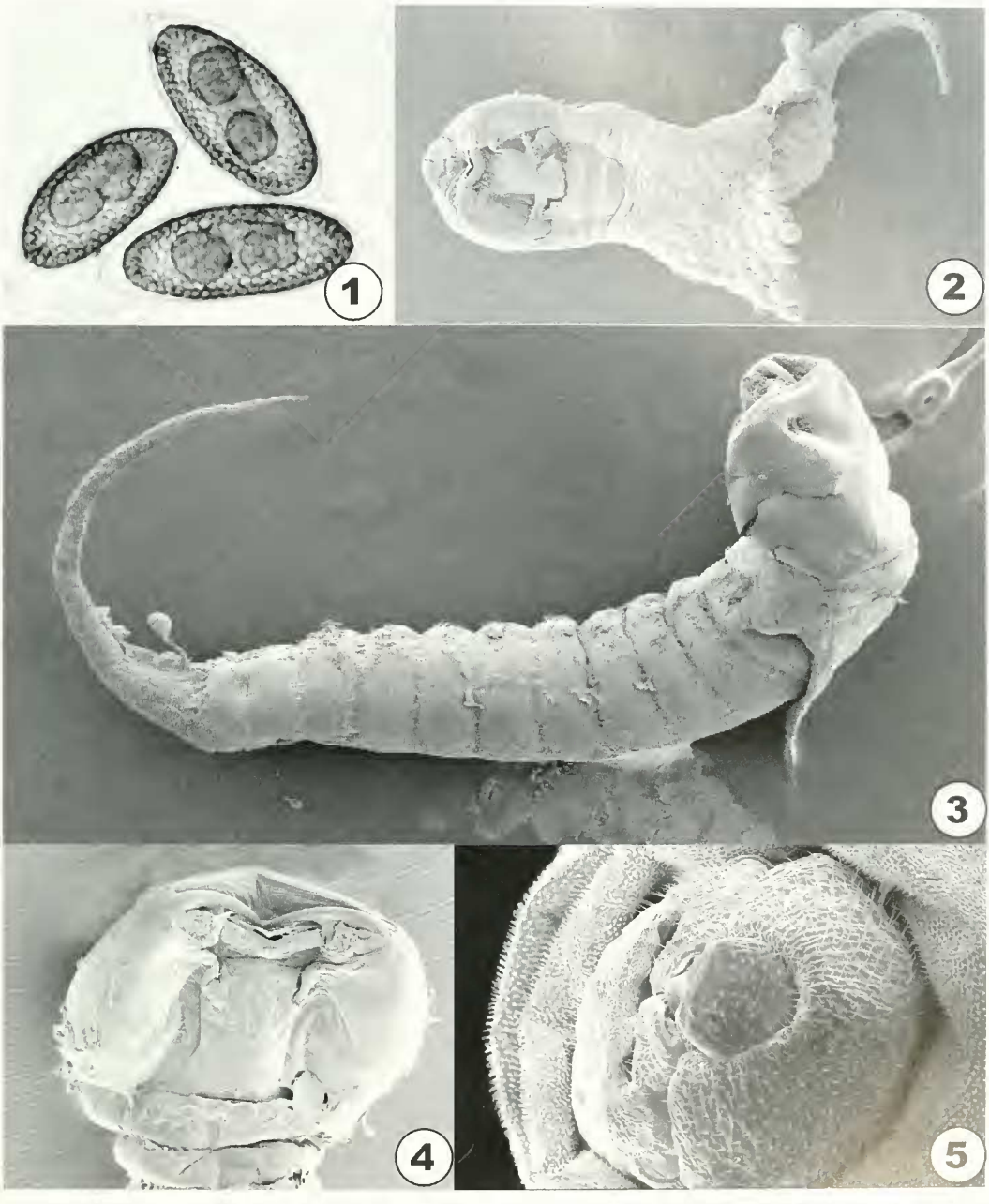
MATERIALS AND METHODS

Periodically, during summer and autumn 1998 and 1999, adults and nymphs of *N. viridula* were collected in the fields in Umbria, Lazio and Sicily regions following the seasonal sequence of host plant species. Most of the specimens were collected on maize and various vegetable plants. A study colony of the parasitoid was established and maintained in Italy by GS. Parasitoids were reared in the laboratory at a temperature of 24 plus or minus 1 degree C, relative humidity 65% plus or minus 5%, and light/dark conditions of 16 hours light and 8 hours darkness daily. The insects were kept in plastic boxes and fed with vegetables and sunflower seeds. Boxes were examined daily to collect parasitoid cocoons. Also, each day 5 *N. viridula* nymphs of the same age (reared in the laboratory) were exposed to a parasitoid female in a plastic box (7 × 5.5 × 2.5 cm) for 24 h. The parasitized nymphs were removed from the box and kept separately until appearance of parasitoid cocoons.

Some nymphs were dissected to observe the different developmental stages of the parasitoid. For SEM analysis the immature stages were fixed in Karnovsky's medium (Karnovsky 1965) for one hour at 4 °C, dehydrated in graded ethanol series, critical-point dried, mounted on stubs, coated with gold and observed with a Philips EM 515 scanning electron microscope. Adult specimens were preserved in 95% ethanol and sent to SRS for description. Preserved adult specimens were transferred to 100% ethanol for 24 hours, then into chloroform for 30 minutes prior to drying and point-mounting to prevent shrinkage.

Aridelus rufotestaceus Tobias, 1986
(Figs. 1–10)

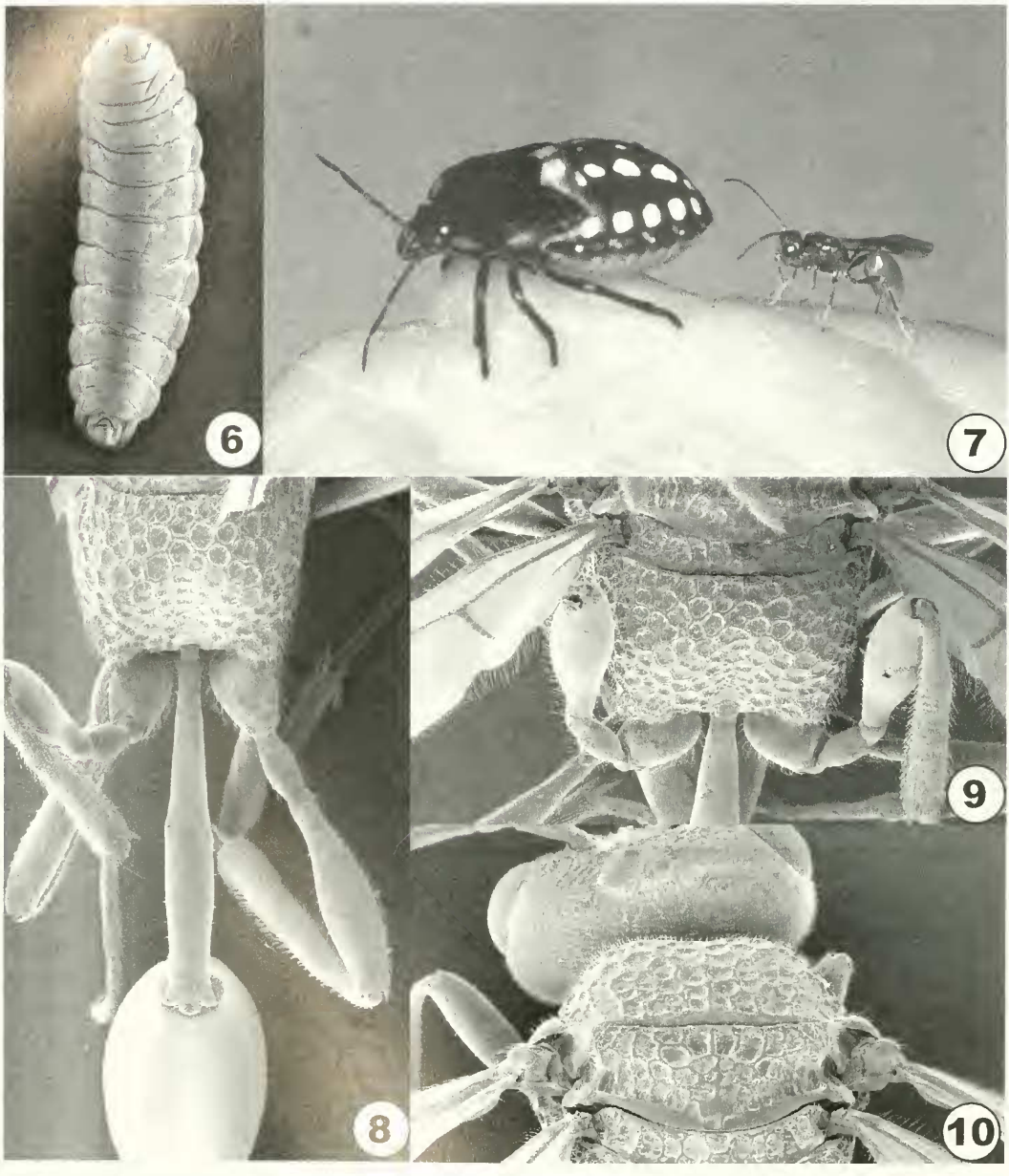
Description of adult female.—length of body 4.8 mm; length of fore wing 3.5 mm. *Head*: Width of head in dorsal view 2.1 times its length; length of first flagellomere 1.5 times length of second flagellomere; length of first and penultimate flagellomeres 4.5 and 0.8 times their width, respectively; median frontal carina weakly developed and somewhat obscured by coarse punctate sculpturing; vertex sculpture densely punctate; ocellar-ocular distance 4.5 times ocellar diameter; occipital carina dorsally well-developed and complete; length of eye in dorsal view 1.6 times length of temple; face and clypeus scultpture densely and coarsely punctate; intertentorial line 1.6 times length of tentorial-ocular line; malar space 0.33 times height of eye. *Mesosoma*: 1.8 times longer than wide in dorsal view, densely areolate. *Wings*: Pterostigma 2.0 times longer than wide at midpoint, anterior margin distinctly rounded and protruding well beyond anterior margin of wing as delimited by vein C+SC+R; length of marginal cell 0.85 times pterostigma length; vein r nearly perpendicular to pterostigma and 2.5 times longer than vein 3RSa bordering second submarginal cell dorsally; vein 3RSb nearly straight basally then curving towards wing margin apically; vein m-cu slightly antefurcal relative to vein 2RS, with very short segment of vein (RS+M)b present. *Metasoma*: Entirely smooth and highly polished; length of first metasomal segment 7.0 times its width at spiracles; metasoma beyond petiole 2.7 times longer than wide in dorsal view; ovipositor sheath very short, exposed portion about



Figs. 1–5. *Aridelus rufotestaceus*. 1, Eggs with developing embryos, 200×. Figs. 2–4. First instar larva. 2, Larva still partially surrounded by trophamnion and teratocytes, ventral view, 70×. 3, Lateral view, 100×. 4, Ventral view of head capsule and mouthparts, 170×. 5, Third instar larva, antero-ventral view of head capsule and mouthparts, 105×.

0.5 times length of hind basitarsus. *Color*: Head, antenna basally, lateral borders of pronotum, legs, and metasoma orangish brown; mandible apically, ocellar triangle, remainder of mesosoma, and ovipositor sheath black; wing venation brown, membrane clear to slightly dusky medially.

Variation.—Body position at death vary-



Figs. 6–10. *Aridelus rufotestaceus*. 6, Third instar larva, ventral view, 11×. Figs. 7–10 Adult. 7, Female near *Nezara viridula* nymph. 8, Propodeum and metasoma, dorsal view, 42×. 9, Propodeum, dorsal view, 38×. 10, Head, mesoscutum, and scutellum, dorsal view, 42×.

ing from metasoma fully extended posteriorly to fully extended anteriorly (ovipositional stance) with metasomal petiole bent under mesosoma and apex of metasoma extending well beyond face. Some individuals appear darker with the head dorsally, hind femur, hind tibia, petiole, and dorsum of metasoma posteriorly more or less infused with smokey black pigmentation. In all cases dead preserved specimens appear somewhat to have a darker mesosoma; while alive some or-

angish brown color shows through the darker black pigmentation. Aside from genitalic differences, the male is quite similar in form and sculpture, but is much lighter in color appearing mostly orange, even over the mesosoma where black pigmentation is limited to smokey pigmentation along the borders of the areolation.

Description of immature stages.—The egg is alecithal (with no visible yolk), oval, with a clear chorion through which the white embryo and developing trophamnion are visible. The developing embryo has a large oval head capsule, followed by 12 similar undifferentiated body segments. The thoracic segments are not visibly different from the abdominal segments. The trophamnion forms a large mass of spongy white teratocytes below the embryo, enveloping the embryo posteriorly. The mature embryo has a thick round head capsule with no trace of eyes or antenna, deep anterior tentorial pits, long sickle-like mandibles, simple mouth opening, 11 undifferentiated similar body segments, and 12th segment longer bearing anus ventrally and a long tapering caudal appendage. The first instar larva is of the caudate form, similar to the mature embryo with a thick round head capsule with no trace of eyes or antenna, deep anterior tentorial pits, long sickle-like mandibles, simple mouth opening, 11 undifferentiated similar body segments, and 12th segment longer bearing anus ventrally and a long tapering caudal appendage densely covered with short, thick, flexible setae. The first instar has an apneustic respiratory system, with no visible spiracles. The body becomes much thicker as the young larva feeds and grows. The second instar larva becomes hymenopteriform remains apneustic. The sclerotized head capsule is much smaller, with short mandibles, and becomes enveloped by the fleshy first thoracic segment as the larva grows. The second instar larva is yellowish white with undifferentiated segments, less distinct than in the first instar, and about 5x longer than wide. The caudal appendage is lost. The third and final instar larva is also hymenopteriform and apneustic, but thicker and more maggot-like. It is tapering at both ends and thickest medially, being about 3x longer than wide at maturity.

Biology.—*Aridelus rufotestaceus* was found for the first time in October 1998 near the Umbria region of Perugia, Italy (parasitism rate 4.3%). During the summer of 1999 we found the parasitoid in the Lazio region (parasitism rate 21.7%) and in Sicily (parasitism rate 12.5%). The mature egg is usually lemon-shaped, with a pedicel. When the first instar larva hatches from the egg, the teratocytes dissociate into the hemolymph and increase in size. In laboratory conditions the period from egg deposition to emergence of the mature larva was 23.18 plus or minus 2.77 days (n = 37). The mature larva emerges from the host through a hole in the intersegmental membrane between the ultimate and penultimate segments, crawls away, and spins an oval white silk cocoon. After emergence of the parasitoid larva the host may survive for several days (although clearly not in healthy condition). Adults emerged from the cocoon in 22.27 plus or minus 1.45 days (n = 37). The adult life span was, in mean, 212.08 plus or minus 8.18 days (n = 106) with a range from 6 to 43 days. Reproduction is parthenogenetic (thelyotokous, or sometimes deuterotokous). In the lab only 3 males were obtained relative to 200 females. Prior to oviposition females approach potential hosts on foot and inspect them, both visually and via antennation. Oviposition is typically very rapid, lasting a few seconds at most, during which the female rapidly approaches the host on foot, throws back the antennae, flexes the metasoma under the mesosoma while both exserting the ovipositor and telescoping posterior metasomal segments. Eggs are inserted into the membranous cervical region between the head and thorax or into the intersegmental areas of the posterior abdominal region of

the host. Supernumerary eggs of larvae were dissected from hosts collected in the field and in hosts parasitized in the laboratory, but in all cases only one larva developed per host. In the laboratory *Aridelus rufotestaceus* was able to parasitize 2nd, 3rd, and 4th instar host nymphs, as well as adults. Younger instars were more suitable for parasitoid development with 95% of 3rd instars parasitized and 85.7% of 2nd instars parasitized permitting complete parasitoid development. The highest mortality rate recorded for parasitized adults was 80.0%.

Discussion.—Terminology used in the description follows that of Sharkey and Wharton (1997). This species is a typical member of the genus and can be keyed to genus without difficulty using the key provided by Shaw (1997). This species can be identified using the key to Chinese *Aridelus* species provided by Chen and van Achterberg (1997). In the key to world species of Papp (1965) this species keys to couplet 23, *A. nigrithorax* Muesebeck, but *A. rufotestaceus* can be distinguished from that species by its lighter colored antenna (flagellum entirely black in *A. nigrithorax*), more coarsely sculptured head (head only finely punctate in *A. nigrithorax*), and weakly developed median frontal carina (strongly developed in *A. nigrithorax*). *A. rufotestaceus* can be distinguished from *A. egregrius* Schmiedeknecht, the only other European species, by its more coarsely sculptured head and lighter body (head mostly smooth and body black in *A. egregius*). The three described North America species, *A. fisheri* (Viereck), *A. melanderi* (Brues), and *A. nigrithorax* Muesebeck, are identical morphologically but differ only in color (entirely orange, black head, or black mesosoma, respectively). Given the wide range of color variations seen in *A. rufotestaceus*, it would seem questionable to separate *Aridelus* species based on color alone. Careful field studies are needed for the North American species to examine if the observed color forms are related to patterns of host use.

The study of Čapek and Davidová-Vilimová (1978) suggested that there are four larval instars in *A. egregius*, but our observations suggest only three larval instars in *A. rufotestaceus*. Čapek and Davidová-Vilimová defined their instars 1 and 2 as morphologically similar, but differing only in slight differences in the length of the mandible. In fact, since their first instar was arbitrarily defined as comprising the smallest individuals, and was based only on 2 individuals (N = 2), their sample size was simply too small to demonstrate a statistically significant difference between their instars 1 and 2. Another possible explanation is that all their individuals with long, fighter-type mandibles and caudal appendage belong to the same instar (1) and there are only 3 instars.

It is worth stressing that in Italy no braconid has ever been recorded as parasitoid of Pentatomidae. Moreover, since 1989, the Department of Arboriculture and Plant Protection of the University of Perugia periodically collected *N. viridula* from the field to assess the parasitization level of tachinid flies, and the presence of any braconid was never observed. In consideration of this, we can hypothesize a recent fortuitous introduction of *A. nezaraphagus* in Italy, as happened in the past for the tachinid *Trichopoda pennipes* F. (Colazza *et al.* 1996). Since the parasitoid was already recorded from China and Russia, this may be a natural range extension from eastern areas. Another possibility is that the parasitoid may have previously been present but made a recent host-switch from other hosts. However, no alternate hosts have yet been found in Italy, although the following pentatomids have been examined for the presence of the parasitoid: *Eurydema oleraceum* (L.), *Eurydema ventrale* (Klt.), *Eurygaster* sp., *Graphosoma lineatum* (L). and *G. semipunctatum* (F.).

Material examined for re-description of adult.—3 females: Italy, Palermo, lab

reared ex. *Nezara viridula*, December 1999; 5 females, 1 male, same data except Perugia, October 1998; 21 females, same data except Perugia, August 1999. Specimens deposited in University of Wyoming Insect Museum, Laramie; Nationaal Natuurhistorisch Museum, Leiden, The Netherlands; and Natural History Museum, Budapest, Hungary.

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